New frontiers for anti-biofilm drug development

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ABSTRACT
Pathogenic microbial biofilm, a consortium of microbial cells protected by a self-produced polymer matrix, is considered a worldwide challenge due to the inherent antibiotic resistance conferred by its lifestyle. Living, as it does, in a community of microbial organisms in a clinical situation, makes it responsible for severe and dangerous cases of infection. Combating this organisation of cells usually requires high antibiotic doses for a prolonged time, and these approaches often fail, contributing to infection persistence. In addition to therapeutic limitations, biofilms can be a source of infections when they grow inmedical devices. The challenge imposed by biofilms has mobilised researchers in the entire world to prospect or develop alternatives to control biofilms. In this context, this review summarises the new frontiers that could be used in clinical circumstances in order to prevent or eliminate pathogenic biofilms.

1. Introduction

Treatment of infections has become a worldwide challenge due to the development of antibiotic resistance among microorganisms, especially when resistance at cellular levels and at community level occur together (Fig. 1). Cellular antibiotic resistance, also referred to as conventional resistance, may occur when antibiotic targets are modified, microbial enzymes inactivate antibiotics and microorganisms prevent or reduce the antibiotic accumulation in their cells (Blair et al., 2015). Resistance observed in a community of microorganisms, known as biofilms, takes place when microbial cells aggregate (Bjarnsholt et al., 2013; G. Zhou et al., 2015; L. Zhou et al., 2015). Resistance to antibiotics can be even higher when single cells that present conventional resistance form a biofilm.

Biofilms consist of one or more microbial species, which can be in different metabolic states, encased in a self-produced biopolymer matrix composed by proteins, polysaccharides and DNA (Bjarnsholt et al., 2013) (Fig. 1). In clinical environments, this resistant profile can develop on human body tissue surfaces and medical devices (Romling et al., 2014). Antibiotic therapies against biofilms usually require the use of high doses for prolonged time, and they often fail to combat persistent infections associated with biofilms (Beloin et al., 2014). Besides, most available antibiotics have been developed to target planktonicmicrobial cells, leading to a big gap in the biofilm field.

Potential candidates may act in preventing, disrupting, weakening

or killing the microbial community within a biofilm (Bjarnsholt et al., 2013). In the prevention, anti-biofilm compounds may kill the planktonic cell or block biofilm formation by living cells. In the disrupting process, anti-biofilm compounds may destabilise the matrix, making the microbial cells within the biofilms susceptible to antimicrobial and/or host defense mechanisms (Bjarnsholt et al., 2013). In the weakening approach, anti-biofilm agents may neutralise virulence factors or affect processes involved in biofilm formation, such as quorum sensing. In the killing process, anti-biofilmcompoundsmay present a bactericidal action upon microbial cells from biofilm (Bjarnsholt et al., 2013).
In this review, we present different approaches that have been proposed to decrease biofilm formation (Fig. 1). Attempts to fight against these cellular organisations include drug repurposing, peptides and peptide-based composites, a combination of different compounds aiming to target different aspects of biofilm, development of nanomaterials to combat and/or improve the diagnostic biofilm infections and the development of medical devices made with anti-adherent material or functionalised with anti-biofilm compounds.

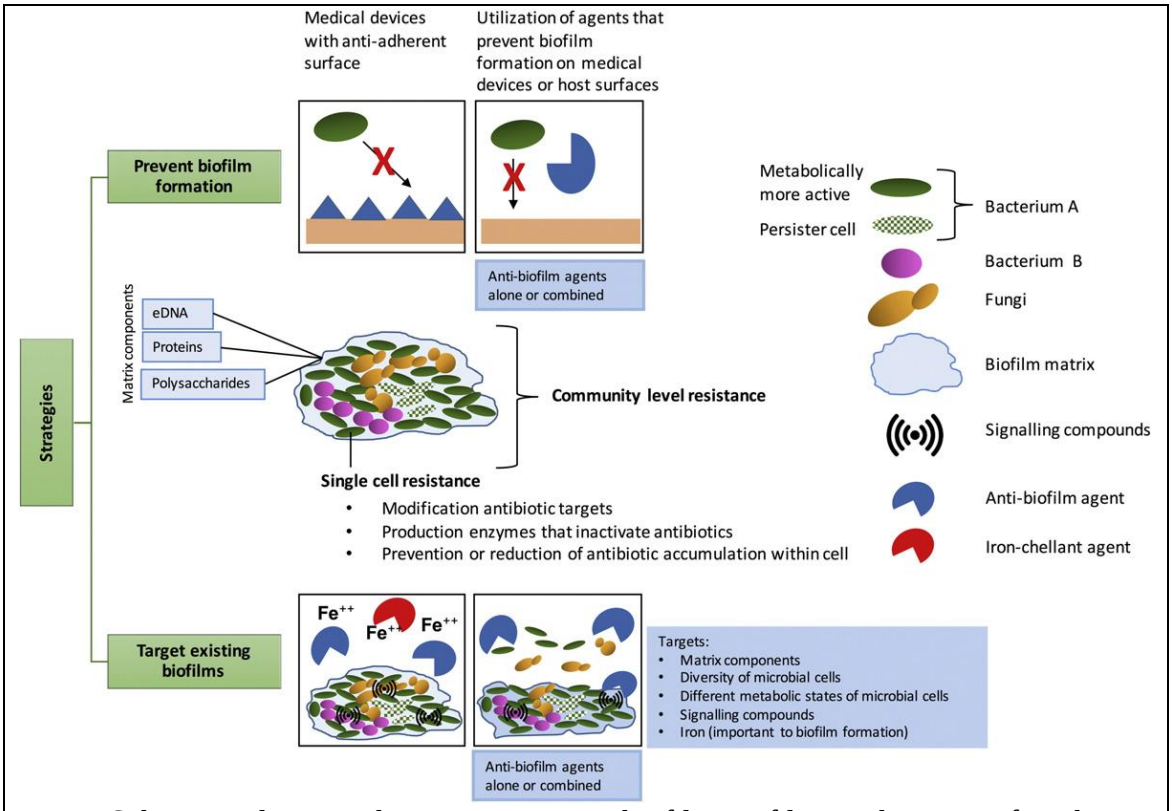

Fig. 1. Schematic diagram showing a common biofilm profile, mechanisms of antibiotic resistance and possible anti-biofilm strategies.

2. The challenge of resistant bacterial biofilms

Currently, bacterial pathogenic biofilms are a remarkable challenge in the medical settings. Biofilm-associated infections are difficult to treat, usually requiring high antibiotic doses (Wu et al., 2015). The concentration of antibiotics to eradicate this bacterial organisation is commonly

higher than that used to inhibit or kill its planktonic counterpart. This resistant life style can overcome host defenses and antibiotic therapies, contributing to the increase of morbidity and mortality in infected patients and consequently increasing hospital costs (Romling et al., 2014). Pathogenic biofilms are normally associated with a number of persistent and chronic infections such as otitis media (Qureishi et al., 2014), periodontal disease (Jhajharia et al., 2015), non-healing wounds and skin infections (Cooper et al., 2014), lung infections in patients with cystic fibrosis (Ciofu et al., 2015), chronic rhinosinusitis (Madeo & Frieri, 2013) and urogenital infections (Zhao et al., 2013). The success of biofilmdevelopment in host tissues could be related to immune defense failure in preventing microbial colonisation or in the elimination of existing biofilms. Otherwise, the prolonged and/or exacerbated response of the host defense against biofilms can damage the host tissue and the neighbourhood, where this microbial community develops, and this may progressively impact the life quality of patients with chronic infections (Beikler & Flemmig, 2011; Zhao et al., 2013; Helwig et al., 2014; Cantin et al., 2015).

Some groups of people present a high risk of developing biofilm infections due to underlying diseases such as diabetes (Mottola et al., 2015) and cystic fibrosis (Ciofu et al., 2015). They become more susceptible to the development of biofilm due to the poor ability of their body to limit biofilm formation. For example, impaired healing of wounds in diabetics may facilitate bacterial development of pathogenic biofilms (Hurlow et al., 2015). Patients with cystic fibrosis have difficulty in coughing up the sputum, making the lung an ideal place for the establishment of biofilm infections (Gupta et al., 2015). Other conditions can facilitate biofilm development, including the exposure of internal body parts to medical devices, such as implants and catheters (Gupta et al., 2015), and poor oral hygiene (Marsh, 2010).

Moreover, biofilms can cause problems beyond the site where the biofilm resides, due to the dispersion of bacterial cells to other parts of the body or through the production of compounds that can trigger other diseases – apart from infections – such as cancer (Johnson et al., 2015) and autoimmune diseases (Gallo et al., 2015). Oral biofilms in dentures, for example, can be a reservoir for pneumonia (O'Donnell et al., 2015). Bacterial cells from biofilm formed in central venous catheters can disperse and cause bacteraemia (Yousif et al., 2015). Interactions between biofilmcomponents such as amyloid curly protein and bacterial DNA, during biofilm formation can trigger an immune activation that induces a pathogenic response in systemic lupus erythematosus, suggesting that amyloids produced by bacteria may contribute to the progression of lupus (Gallo et al., 2015). In addition, pathogenic biofilms have also been detected on the inanimate surfaces of the hospital environment, including in intensive care units, affecting sites such mattresses, curtains and wire clips for holding patients (Hu et al., 2015). In many cases, these biofilms have been formed by multidrugresistant strains (Wiley et al., 2012; Huet al., 2015). Pathogenic bacterial biofilms in natural water and foodmake these environments a potential source of nosocomial and community infections.

Different factors can contribute to the resistant profile of bacteria in biofilms (Bjarnsholt et al., 2013). A high amount of biofilm matrix in mature biofilms for example, can physically limit the antibiotic diffusion within biofilm and consequently reduce the cell killing into biofilm (Holmberg & Rasmussen, 2016). Increased amount of biofilm matrix also can lead to creation of microenvironments with limited amount of nutrients and oxygen gradients, which may contribute to the slow metabolism of cells and consequently to the limited action of specific antibiotics which work better against active growing cells and in oxygenated conditions (Bjarnsholt et al., 2013; Olsen, 2015). Resistance due to reduced metabolism can also be reached by the development of persister cells, a subpopulation of dormant cells (Bjarnsholt et al., 2013). Beyond these factors, matrix components such as extracellular DNA, can bind to antimicrobial agents, like aminoglycosides, and limit the action of antibiotics over microbial cells in biofilm (Wilton et al., 2015).

Individual cells can also contribute to antibiotic resistance in biofilms. Acquisition of antibiotic resistance trough incorporation of foreign genes and/or mutation can lead for example to antibiotic degradation or modification, active efflux of antimicrobial molecules from bacterial cell and modification of antibiotic target (Bjarnsholt et al., 2013). Environmental stress, such as the presence of antibiotic in growth conditions, can induce transitory resistance, regulating for example genes that produce enzymes which degrade antibiotics, alter components of cell membrane (blocking the entry of antibiotics) and upregulate efflux pump (Fernández & Hancock, 2012; Bjarnsholt et al., 2013).

Biofilms in natural environments, including the human body, are usually polymicrobial (Bertesteanu et al., 2014; Swidsinski et al., 2014; Jakubovics, 2015). Biofilm diversity involves the presence of different strains of the same bacterial species, multiple bacterial species or even a mix of bacteria and fungi (Sztajer et al., 2014). In the clinical configuration, this condition can complicate antibiotic therapy. In addition, polymicrobial biofilms have been shown to be more resistant to detergents and to disinfectant, contributing to the maintenance of potential pathogens in the current clinical scene (Sanchez-Vizuete et al., 2015; Vikram et al., 2015). Multiple pathogenic microorganisms may be involved in different antimicrobial targets and, consequently, different antibiotic classes need to be used. Moreover, mixed species of biofilms can be more tolerant to antibiotics than single biofilm species (Lee et al., 2014). Besides, polymicrobial biofilms may cause infections in different systems of the human body or different clinical symptoms, making the diagnosis and antibiotic therapy difficult.

Life in a community, in addition to conferring a defensive strategy against environmental stress on microbial cells (e.g., antimicrobials, host defense, desiccation, detergents, and others), also provides a scenario in which the emergence and spread of resistance can be maximised among individual cells within biofilms, improving both defensive and offensive strategies. Mutation rates are at least 100 times higher in cells within biofilm than in planktonic cells (Conibear et al., 2009), providing an increased possibility of developing antibiotic-resistant mutants.

Proximity between cells from a biofilm facilitates the horizontal transfer of resistant genes and, consequently, the spread of resistance (Madsen et al., 2012). Savage et al. (2013), for example, observed that the horizontal spread of antibiotic resistance in Staphylococcus aureus biofilms is increased by plasmid transfer through conjugations and mobilisation.

3. Old drugs that could be useful to control biofilms

Antibiotics are the currently available drug used in clinical practice to treat these infections (Wu et al., 2015). However, this option is often inefficient, requiring the use of antibiotic combinatory therapy. In recent years, efforts have been driven by academic research groups to discover anti-biofilm agents. Some natural, synthetic or biological (such as bacteriophages) approaches have proved to be promising in combating biofilm infections (Wu et al., 2015). Despite the importance of discovering anti-biofilm agents, the process of putting a new drug on the market can be time-consuming and costly. It is estimated that it takes 15 years to put a new drug molecule on the market and a cost of about US$ 1 billion to bring a single medicine to the market. Moreover, there is little investment in the drug market to treat infectious diseases due to the poor economic returns for the pharmaceutical sector (Chong & Sullivan, 2007).

The advantage of using drugs that are already on the market is mainly because they have already been tested in humans. The safety evaluation, pharmacokinetics and side effects are often known. Thus, the timing and cost associated with carrying out trials for newly identified molecules may be reduced. Identification of new uses for old drugs – also known as drug repurposing or drug repositioning – can reduce the expenses of putting a new medicine on the market by about 40% (Chong & Sullivan, 2007).

Since patents do not cover most part of the approved drugs (9000 out of 10,000 drugs), the possibility of screening new uses for classical drugs is greater (Chong & Sullivan, 2007). This renaissance scenario is particularly interesting from the point of view of finding agents to treat biofilm infections. The studies of biofilm development, biofilm associated infections, diagnosis and treatment are relatively new, and only about 30 years have passed since Professor Bill Costerton observed that chronic infections in patients under implanted medical devices were caused by bacteria growing in biofilms, an antibiotic and host-defense-resistant lifestyle (Lappin-Scott et al., 2014). High doses of antibiotics or antibiotic combinations have been used against biofilm infections; however, in many cases these approaches fail, making patients live chronically with pathogenic biofilms. The formation of biofilms by multi-drug resistant bacteria makes anti-biofilm therapy even more complicated. In order to overcome all these issues, there is an urgent need and demand to develop new antibiotics and also to discover and develop anti-biofilm agents. A faster approach to overcome this alarming situation would be by identifying anti-biofilm activity among existing drugs.

Potential anti-biofilm activity has been found among FDA-approved (Food and Drug Administration) drugs such as anti-inflammatory, anticancer

(Alem & Douglas, 2004; Attila et al., 2009; Naves et al., 2010; Ulusoy & Bosgelmez-Tinaz, 2013; Goggin et al., 2014; Reslinski et al., 2015) and antidepressant drugs (Dean & van Hoek, 2015). Such drugs classes have been tested against bacterial and fungal biofilms. The anti-inflammatory drugs have shown anti-biofilm action against bacterial (Naves et al., 2010; Goggin et al., 2014; Reslinski et al., 2015) and fungal cells. Diclofenac and ibuprofen, for example, limited the biofilm formation of S. aureus and Escherichia coli (Reslinski et al., 2015). Studies with Pseudomonas aeruginosa suggested that the anti-biofilm action of the anti-inflammatory ketoprefen and diclofenac could be due to the interference in quorum-sensing signalling (Ulusoy & Bosgelmez-Tinaz, 2013). Sodium diclofenac, aspirin and ibuprofen have also shown antibiofilm potential against Candida albicans (Alem & Douglas, 2004; Abdelmegeed & Shaaban, 2013). Inmammals, these drugs act by blocking the biosynthesis of prostaglandins (a lipid that plays an important role in the inflammatory response), through the inhibition of cyclooxygenase isoenzymes (Ricciotti & FitzGerald, 2011). Prostaglandins can also be produced by Candida cells in planktonic and biofilm state (Alem & Douglas, 2005). The anti-biofilm mechanism of action of these drugs is unknown in Candida, but it is believed that they might act by interfering in prostaglandin biosynthesis (Alem & Douglas, 2004).

Other categories of drugs, such as anticancer and antidepressant, have also shown anti-biofilm activity against infectious bacteria. For example, the anticancer drug 5-fluorouracil has shown ability in the reduction of biofilm formation of P. aeruginosa, E. coli and S. aureus (Attila et al., 2009). Attila et al. (2009) observed that 5-fluorouracil limits the biofilm formation of E. coli K-1 through AriR, a gene involved in the global regulation of biofilm and acid resistance. The antidepressant maprotiline inhibited Franciella novicida biofilm via signalling through 2-component systems (QseC-dependent) (Dean & van Hoek, 2015).

A combination of non-antibiotic FDA-approved drugs with existing antibiotics is an approach that could be useful in preventing biofilm formation. Here, Moreau-Marquis and colleagues showed that the FDAapproved iron chelators deferoxamine or deferasirox with tobramycin, (an antibiotic) kill existing biofilm of P. aeruginosa and limit biofilm formation on cystic fibrosis cells (Moreau-Marquis et al., 2009). Tobramycin and FDA-approved iron chelators eliminate P. aeruginosa biofilms on cystic fibrosis cells. Since iron availability is important for biofilm formation, the authors suggested that the limitation of this metal induced by the iron-binding activity of those iron chelators might contribute to increasing the action of tobramycin to inhibit or disrupt biofilm formation (Moreau-Marquis et al., 2009).

Although drug repurposing seems to be a promising approach to prevent or combat biofilm infections, it is important to consider that the ideal anti-biofilm candidate would be a drug that is off-patent, safe and works for this purpose within the maximum recommended therapeutic dose for an already-approved indication — this principle is applicable for repurposing of candidates for any type of therapy (Oprea & Mestres, 2012).

4. Peptides and other designed

compounds with anti-biofilm activities

Due to the clinical challenges in combating pathogenic biofilm by antibiotics, that are currently on the market, researchers worldwide have been exploring new anti-biofilmtherapeutic options.Most studies involving the currently available antibiotics used in the treatment of (acute or chronic) infections have been based on experiments with planktonic (free-floating) microorganisms (Bjarnsholt et al., 2013).

The exploration of compounds to control chronic infections strengthened after the association of these infections with biofilms. Currently, awide numbers of compounds, natural or synthetic, have been evaluated as an option to combat pathogenic biofilm in several clinical fields.

In this context, peptides and designed peptide-based compounds, such as peptidomimetics, have emerged as potential anti-biofilmagents (Mojsoska & Jenssen, 2015).

Initially, in the context ofmicrobial infections, peptides from natural sources (vertebrates, invertebrates, plants, fungi and bacteria) have been widely explored for their direct antimicrobial potential against different pathogens, including bacteria, fungi, viruses and protozoa (Mojsoska & Jenssen, 2015). Later, some natural peptides were shown to be better for immunomodulatory than antimicrobial activities in physiological conditions found in vivo (presence of monovalent and divalent cations, serum and anionic polysaccharides) — improving the host defense response in an infection state (Fjell et al., 2012). The term “antimicrobial peptides” (AMPs) has been suggested when direct antimicrobial activity is observed and the term “host defense peptides” (HDPs) has been indicated when immunomodulatory functions are explored (Fjell et al., 2012).

Currently, more than 2000 AMPs isolated from different living organisms are catalogued in the Antimicrobial Peptide Database (Wang et al., 2009). These peptides can be classified based on different characteristics, including length (less than 100 amino acid residues), net charge (cationic, anionic or neutral), structure (usually β-sheet, α-helix, extended, loop) and hydrophobicity (hydrophobic, amphipathic and hydrophilic). Such characteristics can influence the activity and mode of action of these peptides (Bahar & Ren, 2013), and they have been explored as a way to develop improved peptides. Improvements involve making these peptides into therapeutically suitable drugs, through optimisation and design, in order to increase their antimicrobial or immunomodulatory activity, reduce their toxicity and size (minimising the costs of manufacturing) and make them resistant to proteolytic degradation by host proteases. A common way to do this is by substituting, inserting or deleting specific amino-acid residues of natural HDPs. Another way is to develop peptidomimetics, such as antimicrobial peptoids, compounds based on peptides that present altered backbone or non-natural or rare amino acids, which are changes uncommon in nature (Mojsoska & Jenssen, 2015).

Nowadays, in the “biofilm era”, many of those peptides (natural or synthetic antimicrobial and/or immunomodulatory) and antimicrobial peptidomimetics have proved to have action againstmicrobial

biofilms (Mansour et al., 2015; Mojsoska & Jenssen, 2015). Currently, optimisation and design have been directed towards the development of anti-biofilm peptides/peptidomimetics. These compounds have been acclaimed as potential agents to combat biofilm in several clinical scenarios, including oral and wound sites, and on medical devices (Harding & Reynolds, 2014; Mansour et al., 2015; Mojsoska & Jenssen, 2015; Wang et al., 2015). They have shown ability in preventing biofilm formation, dispersing existing ones, reducing biomass and/or killing microbial cells within biofilms (Segev-Zarko et al., 2015).

Some peptides at specific concentrations are able to limit biofilm formation without affecting planktonic growth. For example, the natural peptide LL-37, its synthetic analogue 1037 (de la Fuente-Nunez et al., 2012), the immunomodulatory peptide IDR (innate defense regulator)-1018 and the D-enantiomeric peptides DJK-5 and DJK-6 are able to reduce or prevent biofilm formation at concentrations lower than the MIC (minimal inhibitory concentration) — i.e., the concentration used to inhibit the growth of planktonic cells (de la Fuente-Núñez et al., 2015; Ribeiro et al., 2015). This feature supports the idea that anti-biofilm activity is independent of antimicrobial activity (de la Fuente-Nunez et al., 2012). Interestingly, the anti-biofilm effect on pre-formed biofilms has also been shown to be concentrationdependent. For example, 0.8 μg.mL−1 of the peptide 1018 induces cell dispersion of P. aeruginosa biofilms, while 10 μg.mL−1 of this peptide triggers cell death within biofilms. Peptidomimetics, such the antimicrobial peptoid 1 and 1-C134mer, have shown an ability to eliminate existing P. aeruginosa biofilm at their MIC (Liu et al., 2013). Since the eradication of existing biofilm by conventional antibiotics usually requires doses much higher than their MIC (in some cases up to 1000 times) (Hoiby et al., 2010), such antimicrobial peptoids could be a potential agent to treat persistent infections associated with biofilms.

Studies have shown that some peptides exert their anti-biofilm activity through interference in the expression of genes involved with biofilm formation. The peptide LL-37, for example, downregulated genes associated with the assembly of flagella, which is important for adherence during biofilm development, and with quorum-sensing signalling of formation of P. aeruginosa (Overhage et al., 2008). Besides, LL-37 upregulated genes involved in twitchingmotility, a formof translocation over moist surfaces independent of flagella and mediated by the type IV pili (Mattick, 2002; Overhage et al., 2008). This could lead bacteria to move across the surface, thus limiting the formation of structured biofilms, like mushroom structures, as usually observed in flow cell experiments (Picioreanu et al., 2007). Recent studies have shown that the anti-biofilm activity of the peptide IDR1018 and of the D-enantiomeric peptides DJK-5 and DJK-6 could be associated with the degradation or prevention of accumulation of (p)ppGpp (guanosine pentaphosphate), an intracellular signal molecule involved in the formation and maintenance of biofilms. Thus, peptides must cross the cell membranes and affect intracellular targets, such as (p)ppGpp, to prevent or disrupt biofilms.

The therapeutic use of improved peptides and/or peptidomimetics as anti-biofilm agents seems promising, since some of them can resist host conditions such as enzymatic degradation (Han et al., 2015; Wang et al., 2015), like those present in saliva (Wang et al., 2015) and physiologic ion levels (Choe et al., 2015). In addition to these characteristics, some peptides present immunomodulatory functions, which can improve the host response againstmicrobial cells. The peptide IDR1018, for example, at a concentration of 10 μg.mL−1, prevents biofilm formation and substantially kills cells within oral multispecies biofilms in the presence of human saliva (Wang et al., 2015), which can reduce peptide activity, as observed for human cathelicidin LL-37 (Bucki et al., 2008). With concentrations lower than 20 μg.mL−1 (a concentration that did not affect planktonic cell growth) 1018 inhibits a broad spectrum of bacteria (de la Fuente-Nunez et al., 2014), including ESKAPE pathogens (that include Enterococcus faecium, S. aureus, Klebsiella pneumoniae, Acinetobacter baumannii, P. aeruginosa and Enterobacter species), considered by the Infectious Diseases Society of America to be the cause of the majority of hospital infections (Pendleton et al., 2013). Besides, a nontoxic effect of 1018 against human fibroblasts (200 μg.mL−1) (Steinstraesser et al., 2012) and erythrocytes (100 μg.mL−1) (Steinstraesser et al., 2012) was observed at concentrations higher than those used to demonstrate anti-biofilm activity. The peptide IDR1018 was also able to acceleratewound healing in S. aureus infected non-diabetic pigs in a concentration-dependent manner, when compared to a vehicle-treated control (Steinstraesser et al., 2012). Since Staphylococcal biofilms impair wound healing, as observed in a wound healing murine model (Schierle et al., 2009), the peptide IDR1018 could be a promising agent to treat cutaneous infection by disruption of the biofilm.

5. The potential of synergistic drugs

Microbial biofilms have been linked to a range of chronic infections, such as urinary, pulmonary, wound and prosthetic joint infections (Romling et al., 2014). The therapy to eliminate existing biofilms usually requires high and prolonged antibiotic doses, and this often fails to eradicate the biofilm infection. In many cases, combinatory antibiotic therapies are needed to eradicate biofilm infections (Wu et al., 2015). Synergistic antibiotic combinations present several advantages: expanding the antimicrobial and anti-biofilm spectrum, further reducing the cytotoxicity due to the use of lower doses of drugs, and preventing the emergence of resistant bacterial mutants during therapy (de la Fuente-Nunez et al., 2014).

Considering that biofilms resist antibiotic therapies through multifactorial mechanisms (Bjarnsholt et al., 2013), combining strategies could promote improved treatment of biofilm-related infections. Combined strategies could promote synergy due to interference in different biofilm targets, such as matrix components, persister cells (dormant microbial cells highly tolerant to antibiotics) and microbial diversity. Current studies to access anti-biofilm synergy between compounds involve agents with known and unknown mechanisms of antibacterial

and anti-biofilm activity. Combinatory experiments with agents that present known action mechanisms include those that act in single cell targets, for example: membranes, efflux pump, protein synthesis, or that act on biofilmcomponentmatrix (e.g., extracellular DNA and polysaccharides) or signalling molecules (Bjarnsholt et al., 2013).

Since a number of different compounds present anti-biofilm and/or antimicrobial action, the possibility of constructing diverse groups combining themis promising. Theways to test potential combinatory drugs include antibiotics already on the market and other compounds, such as natural or synthetic peptides, peptidomimetics, enzymes (e.g., DNases), bacteriophages (Chan & Abedon, 2015), essential oils, secondarymetabolites (Kunze et al., 2010), Food and Drug Administration (FDA) nonantibiotic drugs and others (Moreau-Marquis et al., 2009; Dean & van Hoek, 2015). Many of these compounds have shown higher activity in inhibiting or targeting existing biofilms when combined.

Strategies to access synergy against biofilms involve predominantly a combination of two antibiotics and antibiotics with a remarkable diversity of potential anti-biofilm compounds. Some studies have also explored combinations between antibiotics and FDA non-antibiotic compounds, as way of repurposing drugs (Moreau-Marquis et al., 2009). Successful combination therapy could have a positive outcome, using a combination of immunomodulatory (in order to improve the host response against biofilm infections) and anti-biofilm agents. However, the use of combinatory approaches with compounds that can modulate the immune system could be difficult, since this strategy requires more laborious experiments to assess immune response and microbial load (in vitro approaches using co-cultures of mammalian cells and microbial cells or in vivo experiments).

Although in clinical practice the combination of antibiotics against biofilm infections is predominant, in many cases this strategy may have a tendency to fail (Wuet al., 2015), may be because the antibiotics used are ineffective against biofilmorganisation. Resistance to a combination of antibiotics could be due the usual polymicrobial nature of biofilms, to multi-drug resistance to antibiotics of some cells within the biofilm, due to the presence of persister cells or even due to a combination of all these situations. Following this line of thought, it is important to investigate not only anti-biofilm agents, but also to pursue antimicrobial agents that target multi-drug-resistant bacteria, such as methicillin-resistant S. aureus (MRSA) and carbapenem-resistant bacteria, which can reveal new insights about the use of synergetic drugs. In addition, it important to carry out more studies of combinations involving multispecies biofilms in order to target this organisation.

6. Anti-biofilm nanotools

With the increasing number of multidrug-resistant organisms, it is essential to focus on possible therapeutic molecules that can overcome and eliminate this threat. Nanotechnology is one of themost prominent areaswith the potential to tackle almost every aspect of microbial infections (Alves et al., 2010; Cavalieri et al., 2014; Zhu et al., 2014). In recent years, counting bacterial diagnosis, antibiotic delivery and medical devices,

more than 10 nanoparticle-based products have been approved and marketed, which confirms the potential of this area. Even with this potential, and with widespread studies, there are some disadvantages that have not yet been overcome to combat bacterial infections, but many novel products could be used in the future to improve treatment and achieve total eradication.

Nanotechnology has been applied to treat diseases and prevent health issues (Naik et al., 2015). One of the main areas in focus is the development of therapeutic nanoparticles (NPs) for anti-biofilm applications. NPs can be synthesised through many different methods and approaches (Tran & Tran, 2012). The reason why these molecules are so well studied and tested in the therapeutics of infections lies in their properties. Some of themhave shown inherent antimicrobial properties and improved retention time in the body, optimising the capacity to deliver one or more drugs (withminimal side effects and enhancing tissue distribution), reducing the probability of the development of bacterial resistance (Huh & Kwon, 2011; Tran & Tran, 2012). Amongst many examples,metal nanoparticles are themost explored in this field. Metal NPs are mainly constituted by a metal or metal oxide, such as silver (Ag), gold (Au), zinc oxide (ZnO), copper (Cu), iron (Fe) or magnesium fluoride (MgF2). Of these, Ag NPs show the most promising results, due to their high antimicrobial effects on bacteria and biofilms (Li et al., 2008; Ruparelia et al., 2008; Lellouche et al., 2009; Liu et al., 2009; Car et al., 2014; Palanisamy et al., 2014; Salunke et al., 2014; Abdulkareem et al., 2015; LewisOscar et al., 2015; Manju et al., 2016). Their mechanism of action is not yet fully understood, but it is clear that the uptake of Ag^+ ions occurs, followed by cell membrane rupture (Palanisamy et al., 2014). Besides this, researchers have studied the possibility of Ag NPs being attached to glass surfaces, showing that they have high anti-biofilm action and can be applied in novel biomedical devices (Taglietti et al., 2014). Even so, the application of these molecules in clinical settings has some disadvantages that need to be addressed, such as the potential toxicity of long-lasting exposure to metals and the specificity to the target tissues (Huh & Kwon, 2011).

In order to avoid the toxicity problem, the synthesis of polymeric nanoparticles has also been proposed, with a different mode of action from that of metal nanoparticles (Turos et al., 2007). In this case, the NPs would function as drug carriers that deliver the therapeutic molecule into the infected tissue, especially those that are water-insoluble, improving the effect on the biofilm (Turos et al., 2007). Polymeric NPs also include chitosan-coated NPs (Pelgrift & Friedman, 2013). Their antimicrobial effect occurs through chitosan itself, acting at the membrane level and DNA of the bacteria, recruiting immune cells and chelating metalloproteins essential for biofilm growth (Pelgrift & Friedman, 2013).

Major efforts have been made to achieve the specificity of NPs to the target tissue. To this end, different antibody-conjugated NPs have been developed. The antibodies are attached to the nanoparticle surface, which will allow them to combat only the microorganism with the corresponding antigen (Look et al., 2010). These NPs are considered very

promising in the fight against microbial infections due to the flexibility of their synthesis and to their possible function as drug or cytokine carriers, having a greater therapeutic effect. The limitation of these nanomaterials lies in the efficiency of the drug delivery and in the stimulation of the immune response, in the case of the drug and cytokine carriers, respectively (Smith et al., 2013). A more complex system is a combination of metal and antibody-conjugated NPs that was achieved by Norman and co-workers, synthesising gold nanorods with specific antibodies to target bacterial cells (Norman et al., 2008). In this case, gold was chosen due to its ability to absorb near-infrared (NIR) radiation, transferring the absorbed energy as heat to the bacterial surface, inducing irreparable cellular damage. Combining this antimicrobial effect with antibody targeting, it was possible to create a system that has an improved therapeutic effect with great specificity (Fig. 2) (Norman et al., 2008).

Another example of the possible application of nanoparticles to antibiofilm therapeutics is nitric oxide (NO) NPs (Fig. 2). In this case, the nanoparticles work also as carriers of NO, which has an antimicrobial effect on the target cells, releasing reactive free radicals, similar to those produced by the inflammatory cells (Hetrick et al., 2009). The material that is used to synthesise the NPs is required to not react with NO, and is normally silica (Hetrick et al., 2009). Despite the proven advantages that these NPs have shown, toxicity is a major problem, as are the broad-spectrum target cells, raising questions about the possible therapeutic applications. Furthermore, regarding silica NPs, another example includes those with incorporated fluorescent dyes, without a therapeutic effect but more as bacterial cell monitors. In these cases, NPs have nearly 10,000 molecules of encapsulated dyes, which can be of only one type of dye or a system of two or more dyes acting as Förster resonance energy transfer (FRET) pairs (Zhao et al., 2004; Wang et al., 2007). To target the bacterial cells, these NPs have specific antibodies attached to the surface. The possible application of these nanomaterials to therapeutics will help to identify new sites of infection even in the absence of symptoms in the host.

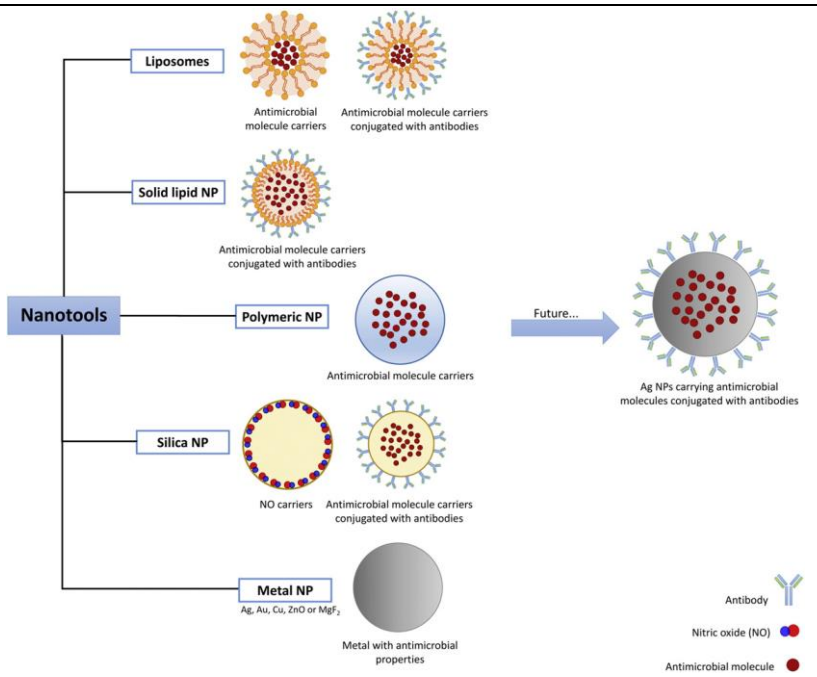

Fig. 2. Schematic diagram illustrating strategies that could be used for the development of anti-biofilm nanotools.

Besides nanoparticles, liposomes and solid lipid NPs have been studied in order to have an antimicrobial and anti-biofilm effect (Muller et al., 2000; Torchilin, 2005) (Fig. 2). Different possibilities for constructed systems are as flexible as nanoparticles,with the difference that they are constituted by lipids, which have the advantage of being more biocompatible than metals, silica or polymers. Also, the incorporation of the drug is usually higher,which increases its therapeutic effect; nevertheless, the low retention time can imply higher doses or more doses over time (Muller et al., 2000; Torchilin, 2005).

Clearly, nanomaterials have a great potential for the future of biomedical therapeutics, with a large number of possible formulations to be introduced to the pharmaceutical market. However, until now, only a few nanomaterials targeting infection were approved by FDA (Table 1). Different nanoparticles and liposomes are being developed each month, applied to specific bacterial species or with a broad-range action, having also greater therapeutic action. The future lies in how to overcome the limitations that the systems already developed have shown, such as toxicity, drug release efficiency, specificity, or the ability to fight the multi-resistant pathogens that have evolved on recent decades. In this regard, one possibility is to combine more than one antimicrobial agent, whether encapsulated or present in the NP material (Huh & Kwon, 2011). Incorporating specific antibodies into the metallic NP surface to increase its specificity is just one ofmany examples of how to obtain a promising drug (Norman et al., 2008). Another possibility is to use silver nanoparticles-embedded polymersomes, with ampicillin incorporated in the hydrophilic compartment, which had shown to have promising results against a resistant strain of E. coli (Geilich et al., 2015). Even more ambitious is combining silver nanoparticles (those

that showed the best anti-biofilmresults) (Palanisamy et al., 2014), carrying antimicrobial drugs for delivery (like antimicrobial peptides or commercially available drugs), with antibodies to improve the specificity for the targeting bacteria. This could be a future approach in the design of newNPs (Fig. 2). Themain objective, besides direct therapeutics, will be the use of these improved nanomaterials in a synergistic approach with biomedical devices, in order to overcome the bacterial infections that occur so frequently.

7. Construction of novel biomedical devices

Biomedical devices play an important role in healthcare practice, being essential for the treatment of diverse pathologies (Zhu et al., 2014). Catheters, implants, prosthetic joints or sutures are nowadays used in several clinical treatments, but their application is associated with an increase in risks of infection. Nearly 45% of infection pathologies are device-related, involving bacterial adhesion to the device and subsequent proliferation and biofilmdevelopment (Romling et al., 2014; Zhu et al., 2014). In order to overcome these biofilminfections, novel protective devices need to be designed in a way that can prevent attachment or that have an inherent antimicrobial activity, promoting degradation, detachment and interference in biofilm regulation and/or development (Romling et al., 2014). In addition, antibiotic resistance has grown in recent decades, making it necessary to fight these super-resistant bacteria that promote device-related infections and that are so difficult to eradicate with the current possible treatment (de la Fuente-Núñez et al., 2013).

The approaches used so far in order to construct devices applied in therapeutics have followed different strategies, but the most well known and studied are: the coating of the material with molecules that have an antimicrobial effect (bactericidal surfaces) or the alteration of surface architecture in order to prevent the attachment of bacterial cells (anti-biofilm surfaces) (Hasan et al., 2013).

In order to create biomedical devices that avoid bacterial infections, bactericidal surfaces have also been developed. Bactericidal surfaces are characterised by the capacity to induce cell disruption and cell death, either by being in contact with bacterial cells or by releasing an effective antimicrobial agent (Hasan et al., 2013). In this case, nanotools developed recently have played a major role, coating the biomedical devices with molecules that have shown the most promising results in terms of therapeutics, like nanoparticles or liposomes (Zhao et al., 2009; Zhu et al., 2014). This type of surface has the advantage of delivering the drugs directly into the infection site, resulting in high local drug doses without exceeding the systemic drug toxicity and preventing possible side effects (Costa et al., 2011).

Naturally, nanoparticles have played an important role here as well, being coated to thematerials by different techniques and being different in constitution, but alwayswith the purpose of reducing the adhesion of bacteria cells, and even bactericidal properties. Silver nanoparticles have been implanted in plastic catheters, and have shown good results in retarding biofilm formation (Roe et al., 2008). Also, in a different

approach to attach the NPs to the surface, Tran and collaborators have incorporated Ag NPs on a hydrophobic polymer in situ, trying to improve the silver ion release and taking advantage of the stabilising effect of the polymer (Tran et al., 2015). As an alternative to metal implants, Travan et al. (2011) have designed a polymeric thermoset that functions as a fibre-reinforced composite, using bisphenol A glycidylmethacrylate (BisGMA) and triethyleneglycol dimethacrylate (TEGDMA), formed by Ag NPs stabilised in a lactose-modified chitosan. This complex design had an antimicrobial effect due to the presence of Ag NPs on the surface, resulting from the proximity to bacterial cells. Furthermore, the use of other nanoparticles has also been tested, like copper nanoparticles (Cu NPs). The nanoparticles were incorporated into a nickel layer, forming a film, in order to incorporate copper more efficiently on the surface tested (in this case, a titanium surface) (Vishwakarma et al., 2009). The killing effect on bacteria was due to the release of Cu ions to the environment, but the toxicity of these nanomaterials was questioned. Additionally, following previous work with MgF2 NPs, the therapeutic effect of these nanoparticles deposited on latex-based catheters was evaluated (Lellouche et al., 2009, 2012). The authors coated the surfaces using a sonochemical process, which was shown to be very efficient. However, although the therapeutic action of the NPs seemed promising, it needs to be more extensively tested, and the cytotoxicity to the patient has to be analysed (Lellouche et al., 2012). Finally, still in the possible uses of NPs, chitosan nanoparticles were tested as antimicrobial agents and simultaneously as a remineralising agent of the dentin structure. In this case, chitosan acts as an antimicrobial molecule but also stimulates remineralisation by binding to calcium ions of the dentin structure, through phosphate groups. At the end, the reaction will form a calcium phosphate layer that reconstructs the demineralised structures of the dentin (Carpio-Perochena et al., 2015).

Besides nanoparticles, metals have been used on different surfaces in order to give them an antimicrobial effect to the material. Again, the most extensively studied material is silver. Researchers have tested the incorporation of Ag^+ ions in high-density polyethylene layers that are used in burn dressings (Acticoat™) (Khundkar et al., 2010). In this particular material, the metal is present at the bottom of the layers, allowing a slowrelease,which results in less frequent dressing changes. In another study using silver nitrate ($AgNO_3$), the authors used a photocatalytic activity initiatedwith radiation to forma titanium surfacewith different silver concentrations. The efficiency of the process was tested, butmore studies regarding the cytotoxicity and the antimicrobial activity are needed in order to apply this technique to biomedical devices (Seery et al., 2007). Gallium (Ga^{3+}) was also tested as an antimicrobial agent in biomedical materials. This metal is chemically similar to iron (Fe^{3+}), and this similarity is used to achieve an antimicrobial effect, entering the biological systems of the bacterial cells and replacing the iron ions. The fact that Ga^{3+} cannot be reduced like Fe^{3+}, leading to the disruption of the biological systems, reinforces the potential use of this metal (Cochis et al., 2015).

Table 1 - Examples of United States Food and Drug Administration (FDA) approved drugs considered as nanomaterials against infections (Bawa, 2013). IV—intravenous injection; SQ—subcutaneous injection; SG — subgingival injection; PGLA — poly(glycolide-co-di-lactide); N.A. — not applicable.

Drug	Nanotherapeutic class	Delivery route	Indications	Company	FDA approval
AmBisome*	Amphotericin B liposomes (~45–80 nm)	IV	Fungal infections	ASTELLAS	August 1997
Abelcet	Amphotericin B phospholipid complex	IV	Invasive fungal infections, for those who are intolerant to *	SIGMA TAU	November 1995
Amphotec	Colloidal suspension of lipid-based Amphotericin B (~115 nm)	SQ	Invasive aspergillosis in patients intolerant to *	ALKOPHARMA USA	November 1996
Arestin	Microspheres of PGLA with minocycline hydrochloride	SG	Adult periodontitis	ORAPHARMA	February 2001
Acticoat	Dressings with silver in a nanocrystalline structure	N.A.	Protection of broad-range infections in wounds	SMITH & NEPHEW	May 2005

Alternatively, for the construction of biomedical devices that have a bactericidal behaviour, the potential use of peptides that target different components of the bacteria cells or biofilm has been examined. Lactoferricins, peptides with antimicrobial activity, were conjugated with titanium binding peptides.With the attachment of these peptides, besides the antimicrobial activity that the surface showed, the material inhibited biofilm formation, but more studies have to be done in order to understand the mechanism of the bactericidal activity (Yoshinari et al., 2010). In another study, Li et al. (2015) developed a fusion peptide with the objective of coating biomedical devices and introducing an antimicrobial activity. The fusion peptide is composed of two peptides, where one has antimicrobial activity only at high concentrations (competence-stimulating peptide, CSP), but the surfaces coated have only appeared to present anti-biofilm formation activity, without suppressing bacterial growth (Li et al., 2015).

Synthetically or naturally isolated antimicrobial peptides (AMPs) are themost studiedmolecules used to coat biomedical devices, due to their broad spectrum activity, minimal bacterial resistance, long-term stability and low cytotoxic profile (Costa et al., 2011; Lim et al., 2015). The strategies to coat a surface with AMPs have been well documented and tested, with a great variety of surfaces tested (like polymeric brushes, self-assembled monolayers, metal or even contact lenses) (Willcox et al., 2008). One study immobilised LL-37 (a natural human antimicrobial peptide) by silanisation on a titanium (Ti) surface, using poly(ethylene glycol) (PEG) as a spacer between the surface and the peptide (Gabriel et al., 2006). The authors confirmed the covalent bond of the AMP to the Ti surface, as well as the antimicrobial activity against E. coli (Gabriel et al., 2006). Using the same type of surface, researchers attached an AMP using silane as the chemical linker. The peptide tested, GL13K, had already demonstrated antimicrobial activity in vitro and in vivo against different bacteria strains. In this study, the stability over time was tested when impregnated on a Ti surface, showing promising results (Holmberg et al., 2013). Other researchers used a lactoferricin peptide (hfl1-11) testing two different methods of immobilisation on titanium surfaces. Both of them (physical adsorption and covalent immobilisation) used silane for the attachment of the peptide to the surface, having good results against bacterial cells and biofilm formation (Godoy-Gallardo et al., 2014). Regarding the silicone surfaces, the most used in medicine, tests have been carried out to coat them with antimicrobial peptides after pre-treatment with polymers (PEG),

which served as spacers between the surface and the peptide, achieving good results (Mishra et al., 2013). Gao et al. (2011) also tested the use of polymers to attach AMPs to a quartz surface, assessing antimicrobial activity against bacteria and biofilms in vivo. The results were promising, but the mechanism through which the AMP accomplished the inhibition of bacteria and biofilm development was not clarified (Gao et al., 2011, 2012). Trying to tether two different AMPs to a polydimethylsiloxane surface (PDMS), allyl glycidyl ether (AGE), a polymer, was used as a spacer, binding them covalently. In both cases the surfaces coated showed antibacterial and anti-biofilm activities (Hilpert et al., 2009; Lim et al., 2013). Moreover, different spacers were tested in order to conclude if there is a relationship between the molecules used. The results confirmed that independently of the linker chemistry. AMPs tethered to a cellulose surface are able to induce the death of bacteria only using electrostatic interactions with the biomembranes, without needing to enter the cell membranes (Li et al., 2014).

Another possibility is to immobilise the peptide in self-assembled monolayers, which will function as a linker between the surface and the peptide. To this end, researchers have tested a natural AMP, magainin I, attached to a gold surface, concluding that the antimicrobial activities were maintained, even demonstrating possible anti-biofilm activities (Humblot et al., 2009). In fact, there is a rising number of peptides attached to biomedical surfaces that have shown encouraging results, using spacers for the attachment (G. Zhou et al., 2015; L. Zhou et al., 2015). More studies regarding stability, activity and biocompatibility are necessary before applying themin therapeutics. Among several other examples, AMPs have shown to be the most promising bactericidal molecules in the prevention of biomedical devicesassociated infection. In the future, new natural or synthetic peptides are expected to appear in the pharmaceutical market that could potentially be applied on the coating of these devices.

The use of bactericidal surfaces is known to be effective, but there are many doubts related to specificity and the therapeutic long-term effect after the first contact with physiologic fluids (Campoccia et al., 2013; Hasan et al., 2013). Due to these reservations, the importance of nanotopography in resisting and preventing the cellular attachment was recently recognised, originating a new type of anti-biofilm surface (Truong et al., 2010).

For a surface that naturally repels bacterial cells and that prevents biofilm formation, it is necessary to pay attention to the physicochemical properties, similar to those used to design liposomes for drug delivery. Modification of the surface can occur by functionalisation/derivatisation or by polymerisation (Anselme et al., 2010; Hasan et al., 2013). To functionalise and derivatise the surfaces, it is necessary to consider at least one of the chemical properties, like hydration, hydrophobicity or charge, adapting them to repel the bacteria that are more capable of attachment (Bazaka et al., 2011). A recent study has developed a method that uses microwave radiation to change the native oxide titanium film, promoting an antimicrobial effect. The mechanism by which this occurs is not yet well understood, but the results were promising

(Gopal et al., 2015). Also, some studies have shown that surface roughness (that is associatedwith hydrophobicity) is determinant for bacterial infection, regarding the effectiveness of the attachment (Anselme et al., 2010).

Polymerisation could also be classified as a method that creates bactericidal surfaces, because some of the polymers tested involve the use of an antimicrobial agent that covalently binds the surface, but due to the chemical changes that occur during the process, it can also be considered an anti-biofilm surface (Hasan et al., 2013). As examples of coatings for bactericidal surfaces, in addition to the incorporation of antimicrobial molecules, polymers with functional groups (tertiary amines or N-alamines) or polymers realeasing nitric oxide or oxygen reactive species have also been tested (Nablo et al., 2005; Wang et al., 2011). Focusing on polymers that promote an anti-adherent behaviour in the devices, Yang et al. (2011) showed the potential use of a powerful anchor to activate and increase the efficiency of the surface-initiated polymerisation, using the polymerised version of 2-hydroxyehtyl-methacrylate (HEMA), (PolyHEMA, PHEMA). An additional study showed the possible use of poly(ethylene oxide) (PEO) covalently bonded to the surface as a polymer that reduces the adhesion of bacteria cells. Even so, the study was only tested on a glass surface, needing more tests on other biomedical surfaces (Roosjen et al., 2003). Poly(sulfobetaine methacrylate) (pSBMA) and poly(carboxybetaine methacrylate) (pCBMA), as zwitterionic polymers, also formed polymeric surfaces that are highly resistant to biofilm formation and bacterial accumulation. The authors attributed this resistance to intrinsically strong hydration via electrostatic interactions and hydrogen bond interactions, but tests in medical devices need to be assessed (Cheng et al., 2007). Incorporation of an enzyme (glycoside hydrolase dispersin B, DspB) within a layer-by-layer hydrogel, formed by a polymer (poly-N-acetylglucosamine, PNAG), was likewise tested and showed promising results in inhibiting biofilm formation (Pavlukhina et al., 2012).

Finally, combining methacryloyloxydecyl phosphate (MDP) with PEG, researchers found a new treatment that inhibits the attachment of bacteria to hydroxyapatite (HA) surfaces (used in numerous biomedical devices, like prosthetic and dental implants), testing these polymers also in vivo with positive results (Shimotoyodome et al., 2007). Even with the limitations already shown, the great advantages of polymers are their stability and biocompatibility, which iswhy they are so attractive for use in biomedical devices.

Another example of methods that prevent the formation of biofilms is the surface-active agents produced by microorganisms, known as biosurfactants. These compounds are similar to polymers, acting asmolecules that can disrupt membranes, leading to cell lysis and finally death, or as anti-adhesion molecules that delay biofilm formation (Banat et al., 2000, 2014). In terms of characterisation, they are produced mainly by microorganisms, being amphipathic, which allows themto accumulate at the interface between lipid phases. Lipopeptides (like polymixin and surfactin) and glycolipids (like rhamnolipid and shorolipid) are just two classes of biosurfactants that have shown

their potential to prevent biofilm attachment (Banat et al., 2014; Díaz De Rienzo et al., 2015; Inès & Dhouha, 2015). In the last decade, some authors have tried to develop this type of surfaces, but more studies need to be done in order to properly assess the activity of these devices. One of the studies focused on the pre-inoculation of the material with non-pathogenic bacteria, which acted similarly to a vaccine. Even being a promising result, the non-pathogenic character of the bacteria was questioned (Trautner et al., 2002).

In a different approach, modulating the immune system could be also an alternative to developing biomedical devices against infections. Researchers have shown that nanoscale-coating the surfaces with IL-12, a cytokine that promotes cell-mediated immunity, stimulates the natural body defense system against bacterial infections (Li et al., 2009). The use of bacteriophages, viruses that selectively infect bacteria and promote lysis, has also been addressed with regard to biomedical surfaces, synergistically with the antibiotic ciprofloxacin. Even though they presented promising results against bacteria, the disadvantage of these systems is the bacterial resistance to the phage, which can be manipulated only through genetic methods (Jikia et al., 2005; Carson et al., 2010). Another possibility is to use polymeric matrixes that bind DspB, an enzyme that is able to disperse and detach mature biofilms fromnumerous bacteria strains. The synergistic action of DspB with antibiotics (cefamandole nafate, CFE) or with antimicrobial molecules (like triclosan, a common antiseptic) on catheters coated with them, make this combination a promising strategy to develop materials that have simultaneously antimicrobial (promoted by the antimicrobial molecule) and anti-biofilmactivity (due to the presence of the enzyme) (Donelli et al., 2007; Darouiche et al., 2009).

As observed, numbers of different approaches and/or compounds can be used to develop biomedical devices that aimto control or prevent infectious biofilms. Certainly, promising devices in a medical context would be those that present potent anti-biofilm action and minimal or nontoxic effects on patients that depend on these devices. Studies in this line should also consider this parameter in order to develop new anti-biofilm devices.

8. Conclusion and prospects

Microbial biofilms are a challenge in the biomedical context, especially when they develop on human surfaces or implanted medical devices. Recent intensification of research to understand microbial biofilms and to develop new anti-biofilm therapies, anti-biofilm medical devices and diagnostics tools has provided a hope in this challenging clinical scenario. This has indicated that an integration of different strategies can be successful to combat pathogenic biofilms.Webelieve that a better understanding of microbial biofilms profile associated with optimised resources (anti-biofilm agents, biomedical devices and diagnostic tools) will provide in the future, a fast, inexpensive, potent and safe weapons to combat pathogenic biofilms.

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